



Combination of kinetic maceration - digestion in the extraction of areca seeds (*Areca catechu* L.)

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KEYWORDS

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ABSTRACT

Pinang (*Areca catechu* L.) is one of the plantation commodities from the Palmae group. One of the secondary metabolites contained in areca nut is tannin. Tannins are easily soluble in aquadest so extraction using aquadest solvents is the right choice. Extraction with kinetic maceration method with solvents of distilled water combined with digestion is an effective way to increase extract yield and have a fairly small operational risk. Therefore, in this study studied variations in temperature and extraction time using a combination method of kinetic maceration - digestion of the quality of tannins produced. The experimental design in this study consisted of two factors: the first factor was the extraction temperature with three levels, namely 40; 60 and 80 °C and the second factor was extraction time with five levels, namely 4, 6, 8, 10 and 12 hours. The combination of kinetic maceration - the right digestion on yield and quality of areca nut tannin extract (crude areca nut tannin) was obtained at 40 °C with extraction time of 4 hours with a yield value of 12.20% (dry basic), moisture content of 0.023%, tannin content of 424.99 mg GAE / g. (dry basis) and IC50 42.54 ppm.

Introduction

Indonesia is an agricultural country that has the potential for sizable plantation. One of the results plantation has benefits for the treatment plants are areca. According to data of Central Statistics Agency 2017 production plant areca in 2012-2015 increased from year to year, respectively 42.0; 42.8; 47.0 and 47.1 thousand tons. The areca contains pharmacological activities which include antiparasitic, affects the digestive, nervous and digestive systems, the effects of antioxidant, antibacterial, anti-fungal, anti-allergic, anti-inflammatory, analgesic, and regulate blood sugar, and fat (Gilani et al., 2004; Liu et al., 2013). According to Peng et al. (2015) the seeds of areca nut have 59 compounds that can be isolated and identified that the majority of its compounds are its identifier. Tannin widely used by the pharmaceutical industry, food, leather and furniture which the demand is predicted increase until the year 2025. Tannins have properties can precipitate proteins, absorb metals and minerals. Tannin

compounds in seeds of areca nut can be obtained through the extraction process.

Extraction of tannins from the seeds of areca nut is commonly used methods of maceration and soxhletation (Bhandare et al., 2010; Hamsar et al., 2011; Meiyanto et al., 2008; Mamonto et al., 2014; Sulastri, 2009). Extract yield Areca seed with the method soxhletation and methanol solvent is generated on the research of Mamonto et al. (2014) very small i.e. 3.87%. The small yield and antioxidant activity in the research was suspected due to less effective extraction process used. A study by Sulastri (2009) demonstrated that extraction of the seeds areca nut using a water solvent in maceration extract showing a better results, where the yield of seed areca was 6.089% and tannin content was 6.45%. It was because the tannins are easily soluble in water, thus the extraction using solvent water is the best option. According to Checinel-Filho (2012), extraction method of maceration kinetic with solvent water is an effective way to increase the yield extract. In the dynamic/kinetic maceration, raw materials and

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solvent are retained in the condition of constant stirring. The time of extraction used was shorter (1-12 hours) compared with the extraction time of maceration static (5-14 days), depending on the tool and the stirring rate. Digestion is the process of extraction with kinetic maceration occurring at temperatures of 40 °C to 60 °C for 2-6 hours (Checinel-Filho, 2012). Dynamical movement between solute and solvent as well as increase in temperature between 40-60 °C may escalate the rate of diffusion of material towards the solvent and reduce the time of extraction. According to Maslukhah et al. (2016), the extraction process is affected by several factors include extraction methods, the size of the material, the temperature of the extraction, the extraction time, as well as type and amount of solvent.

Based on above findings, further research on the combination of temperature and the length of time kinetic maceration methods on extraction of

tannin – seeds of areca extracts is critical. This study was aimed to find the best procedure of extraction in producing quality good tannins.

Research Methods

Materials

The main material used in this study were young seed betel areca up to half the old (the color is green to orange) which is obtained from Malang city, East Java. The ingredients in laboratory analysis, among others, aquadest, ethanol, methanol, gallic acid, Na₂CO₃, ethanol, folin denis, DPPH with concentration 0.2 mM in ethanol.

The tools used in the manufacturing process extracts the rough seeds of areca nut is a water bath shaker lap speed 100 rpm, digital scales (Denver Instruments M-310), glassware, Buchner funnel. The tools used in the analysis are digital scales (Denver Instruments M-310), and UV VIS spectrophotometer (20 D Plus).

Table 1. Combination treatment

No.	Extraction temperature (°C)	Extraction time				
		T ₁ (4 hours)	T ₂ (6 hours)	T ₃ (8 hours)	T ₄ (10 hours)	T ₅ (12 hours)
1.	S ₁ (40)	S ₁ T ₁	S ₁ T ₂	S ₁ T ₃	S ₁ T ₄	S ₁ T ₅
2.	S ₂ (60)	S ₂ T ₁	S ₂ T ₂	S ₂ T ₃	S ₂ T ₄	S ₂ T ₅
3.	S ₃ (80)	S ₃ T ₁	S ₃ T ₂	S ₃ T ₃	S ₃ T ₄	S ₃ T ₅

Experimental Design

This research used a Randomized Factorial Design Group with two factors. Factor 1 was the extraction temperature with 3 levels (40, 60 and 80°C) and factor 2 was the extraction time with 5 levels (4, 6, 8, 10 and 12 hours). In total, there were 15 combinations treatment. Each treatment was carried out in triplicate, resulting 45 units of experiments. Combination of experimental treatment can be seen in Table 1.

Research Procedure

Areca nut was sorted to remove dirt or rotten fruit. Next, areca nut was shelled and taken out the seeds. The seeds of areca nut were crushed and dried in a drying cabinet for 24 hours at temperature of 50 °C. Dried areca seed was then crushed and sifted into 60 mesh. Areca seed powder was weighed for 50 g and added with distilled water at volume of 300 ml, then closed using aluminium foil. Following the Kinetic Maceration – Digestion (KMD) (6.8, 4, 10 and 12 hours) carried out at a temperature of (40, 60 and 80 °C) with the speed rotation of 100 rpm. After that, the mixture was filtered using Buchner funnel to get the filtrate seeds. The filtrate seeds was dried using cabinet dryer for 24 hours at temperature of 40 °C.

Then, calculate yield, moisture content, antioxidant activity and levels of tannin. Statistics analysis was performed using ANOVA, Duncan test and Least Significance Difference.

Yield Analysis

Yield analysis was conducted according to Yuwono, dan Susanto (1998) with modification. Raw materials and products were weighed then calculated by dividing the weight of products with raw materials and multiplied by 100%. Yield is calculated based on Eq. (1) and (2):

$$\text{Yield (\%)} = \frac{\text{product weight} \times 100\%}{\text{raw material weight}} \quad (1)$$

$$\text{Yield (\% dry weight)} = \frac{(100 - \text{moisture content})}{100} \times \text{yield (\%)} \quad (2)$$

Moisture Content Analysis

Moisture content analysis was conducted according to AOAC (1999) with modification. Empty crucible is dried in an oven at 105 °C and cooled in a desiccator. Then, the empty crucible was weighed, a sample of 2 g was added into the empty crucible. The crucible containing the sample was placed in an oven at 105°C for 3 hours. Then, the samples were

cooled in desiccator, followed by weighting the cooled samples. Water content is calculated based on Eq. (3):

$$\text{Moisture content (\%)} = \frac{\text{initial weight} - \text{final weight}}{\text{sample weight}} \times 100\% \quad (3)$$

Tannin content

Tannin content was conducted based on Tambe and Rajendra (2014). Prepared 100 ppm gallic acid stock solution, then diluted to 80, 60, 40 and 20 ppm. Next, a standard tannic acid curve was made by entering 0.1 ml of gallic acid solution of various concentrations into a 10 ml measuring flask. Added 7.5 ml of distilled water, 0.5 ml of Folin Ciocalteu reagent, and 1 ml of Na₂CO₃ 35%. Then, added distilled water to the boundary mark and store at room temperature. Measured the absorbance at 725 nm. Then, made a standard curve with the x axis (concentration of gallic acid) and the y axis (absorbance). The blank was 8.5 ml of distilled water, 0.5 ml of Folin Ciocalteu reagent, and 1 ml of Na₂CO₃ 35%. To measure the tannin level of a sample, the procedure was carried out in the same way as making a standard curve, but gallic acid is replaced with a sample of 0.1 ml 1000 ppm.

Antioxidant activity

Antioxidant activity was conducted according Hatano et al. (1998). Measurement of antioxidant activity using the DPPH method was carried out by the following procedure: extract samples were diluted using distilled water at a concentration of 1000 ppm. The extract solution was diluted again to 100 ppm. From the 100 ppm extract solution, a series of extract solution dilutions was made at concentrations of 100, 75, 50, 25 and 0 ppm. Concentrated sample solution then placed in 1 ml of closed test tube. Added 7 ml ethanol solution and 2 ml DPPH 0.2 mM solution. Then, mixed with vortex approximately 1 minute and let stand for 30 minutes. The sample solution in each series of concentrations was measured by its absorbance (A₁) at λ = 517 nm. Absorbance in each concentration series of sample samples was recorded and the linear regression equation was determined in the form of an equation (Eq. 4).

$$y = a + bx \quad (4)$$

Where:

y = absorbance of the sample

a = the regression line intersection on the y axis

b = gradient equation

x = sample concentration

Before that, the absorbance of the control was measured. The control absorbance was a mixture of 8 ml ethanol solution pa with 2 ml DPPH 0.2 mM solution, while the blank was an ethanol solution pa. The absorbance value of the control was recorded. Indigo absorbance control (A₀) was then divided into two (A₀/2) to be included in the IC₅₀ calculation. IC₅₀ values are calculated using the formula = [(A₀/2) - a] / b.

Results and Discussion

Yield

Yield of areca seed extract is the percentage of crude tannin produced from the extraction process seed powder. This yield is calculated in % dry weight. Final form of areca seed extract in this research was in the form of a solid. The yield extract of the seed areca nut from this research is presented in Fig. 1. The results of the analysis of variance (ANOVA) with a confidence level of 95% (α = 0.05) showed that the interaction between extraction temperature and extraction time give significant effects against yield crude seed tannins. In this case, the temperature has no effect, but long extraction time affect the extract yield. This is indicated by the value of the sig smaller than 0.05.

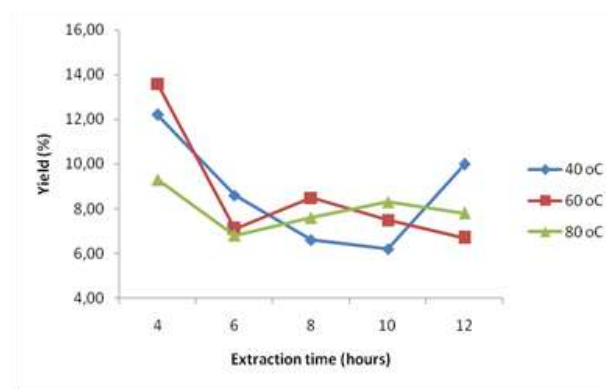


Figure 1. The graph of tannin yield

Extraction time influenced the yield and value as shown in Table 2. An increase in extraction yield showed a decrease in extraction time needed for crude seed tannins. It is suspected that the tannins are dissolved in colloidal water and weak acids. The longer contact with water, tannins will transform into a colloidal (Ismarani, 2012). The ability of tannin to change into a colloid, may be halted as residue during the filtration process, reducing the filtrate yield. The best time needed for the extraction of tannins was 4 hours. It is suspected the tannins can be dissolved and prevent the onset of formation of colloids. Increasing extraction temperature between 40-80 °C does not have an effect on the

value of yield as shown in Fig. 1. This is in accordance with the theory that the methods can be done between 40-60 °C. Increasing temperature up to 80 °C did not increase the yield of crude tannin.

Therefore, the extraction temperature should be in a lower temperature to maintain the quality of crude tannin antioxidant.

Table 2. Yield of crude tannin

Yield of Crude Tannin Areca Seed (% dry weight)						
No.	Extraction temperature (°C)	Extraction time				
		T ₁ (4 hour)	T ₂ (6 hour)	T ₃ (8 hour)	T ₄ (10 hour)	T ₅ (12 hour)
1.	S ₁ (40)	12.20	8.60	6.60	6.20	9.99
2.	S ₂ (60)	13.59	7.10	8.50	7.50	6.70
3.	S ₃ (80)	9.30	6.80	7.60	8.30	7.80

Moisture Content

The result of the moisture content of tannins in this research is presented in Fig. 2. The ANOVA results with a confidence level of 95% ($\alpha = 0.05$) showed that the interaction between extraction temperature and long extraction time was significantly influenced the water content of crude tannin seeds. This is indicated by the value of the sig smaller than 0.05.

Based on Table 3, a higher temperature causes a decrease in water content in crude seed tannins. It is possibly that, when the extraction progresses, the bonds between molecules of tannins with H₂O start

to cut off, thus reducing the bond of intermolecular with H₂O. Thus, it declined the levels of water extract. The same effect was also shown an improved extraction time. Water content decreases with an increasing extraction time. The longer extraction time took place at a certain temperature, then the termination of bonding between molecules and H₂O was greater as a result of a decrease in moisture content. This occasion is called hydrolysis, defines as the termination of water bonding to become their ions. Such mechanism or condition can reduce the amount of water contained in the sample.

Table 3. Moisture content

Moisture Content of Crude Tannin Areca Seed (%)						
No.	Extraction temperature (°C)	Extraction time				
		T ₁ (4 hour)	T ₂ (6 hour)	T ₃ (8 hour)	T ₄ (10 hour)	T ₅ (12 hour)
1.	S ₁ (40)	0.023	0.047	0.026	0.019	0.042
2.	S ₂ (60)	0.031	0.059	0.047	0.063	0.014
3.	S ₃ (80)	0.042	0.047	0.007	0.023	0.023

Tannin content

Tannin content is the amount of mg tannin equivalent to gallic acid every gram of dry weight (mg GAE/g) resulting from the process of extraction of pollen seeds. The tannin content from areca nut are presented in Fig. 3. The ANOVA results with a confidence level of 95% ($\alpha = 0.05$) showed that the interaction between extraction temperature and extraction time had a significant effect on the levels of crude seed tannins. This is indicated by the value of the sig smaller than 0.05.

Table 4 shows that the extraction temperature significantly influenced the tannin content. An increase in the extraction temperature cause a

significantly decrease in the tannin content. Extraction temperature at 80 °C proved to produce the lowest tannin content (253.74 mg GAE/ g dry weight), while a temperature of 40°C produced the highest tannin content (383.68 mg GAE/ g dry weight), in average values. Tannin is a polyphenol compounds that are broken at high temperature. Tannin can be decomposed to pyrogallol, pyrocatechol and phloroglucinol when heated to a temperature of 210°F – 215 °F (98.89 °C – 101.67 °C) (Ismarani, 2012). An increase in extraction temperature is thought to cause part of the tannins to break down.

Extraction time has a significant effect on the tannin content. A longer extraction time can reduce the tannin content. Appropriate test LSD and DMRT results showed that tannin content decreased in 8 to 12 hours of extraction time. It was possibly due to the tannins that are already dissolved in the water is hydrolysed and transformed into a colloidal

formation (Ismarani, 2012). Thus, the tannins clot and bound to another molecule causing it to decline in the concentration. This was also in line with the previous parameters where the yield of crude tannin decreases with increasing of the extraction time.

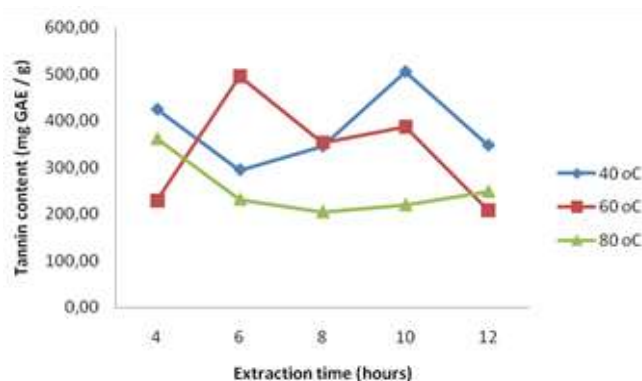


Figure 3. The graph of tannin content

Table 4. Tannin content

Tannin content of Crude Tannin Areca Seed (mg GAE / g. bk)						
No.	Extraction temperature (°C)	Extraction time				
		T ₁ (4 hour)	T ₂ (6 hour)	T ₃ (8 hour)	T ₄ (10 hour)	T ₅ (12 hour)
1.	S ₁ (40)	424.99	294.48	345.46	505.90	347.59
2.	S ₂ (60)	228.62	495.81	353.52	386.78	207.78
3.	S ₃ (80)	362.18	232.30	205.08	220.01	249.12

Antioxidant Activity

Antioxidant activity is shown by the value of the IC50, i.e. the concentration of DPPH remaining (ppm) that does not react with crude seed tannin areca nut as antioxidant compounds. IC50 results from areca seed extract are presented in Fig. 4. The ANOVA analysis with a confidence level of 95% ($\alpha = 0.05$) showed that the interaction between extraction temperature and extraction time does not have significant effects on crude seed tannin antioxidant activity. This is indicated by the value of the sig is greater than 0.05.

Although the antioxidant activity of crude tannin areca nut seeds on this research was not affected by time and extraction temperature, the IC50 value was generated by each extract sample showed a very strong intensity antioxidant (IC50 values of less than 50 ppm) (Jun et al., 2003). Antioxidant in chemical understanding is the compound as donor of the electron. Antioxidant works by donating one electron to oxidant

compounds, thus the activity of oxidant compounds can be inhibited. Antioxidant stabilise the free radicals with complete deficiency of electrons belonging to free radicals, and inhibit the occurrence of a chain reaction of formation of free radicals. In other words, free radicals will react with the donor of an antioxidant compounds forming a more harmless molecule (Winarsi, 2007; Yongsun, 2005).

In accordance with Table 5, crude tannin IC50 value of areca nut seeds at a temperature of 60 and 80 °C have the same trend. Both experiments were seen to have IC50 value that is greater than that of at the temperature of 40 °C. This means antioxidant activity of crude tannin areca nut on low temperature better than at high temperatures. However, if it seen from the intensity, there is no difference from all extracts because the best results was derived from the same intensity range with very powerful activity. It shows that all extracts have best antioxidant activity. At the same concentration, the ability of crude tannin areca nut from all treatment in prevent

the impacts of free radicals is very effective. It can be concluded that the crude tannin areca nut has quite good potential for further application as an antioxidant.

Interaction between extraction temperature and extraction time give significant effects on the levels of crude tannin seed tannins, but it has no significant

effects on antioxidant activity. Although there are differences in the value of tannins from several treatment variations, all tannin extracts have a very active category of antioxidant activity. It means that the lowest tannin extraction results are included in the category of very strong antioxidant activity (less than 50 ppm).

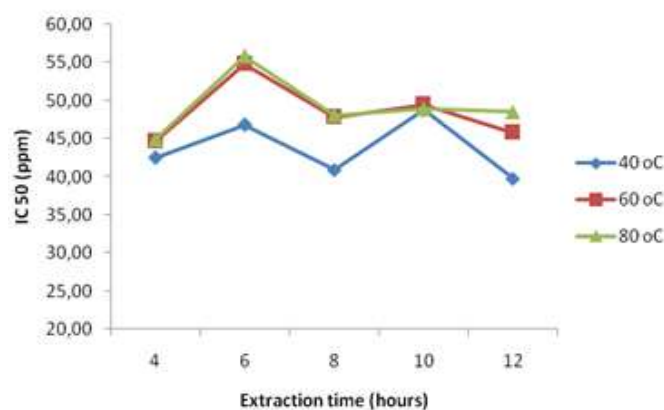


Figure 4. The graph of IC50

Table 5. Antioxidant activity (IC50 value)

		IC50 of Crude Tannin Areca Seed (ppm)				
No.	Extraction temperature (°C)	Extraction time				
		T ₁ (4 hour)	T ₂ (6 hour)	T ₃ (8 hour)	T ₄ (10 hour)	T ₅ (12 hour)
1.	S ₁ (40)	42.54	46.83	40.91	48.82	39.76
2.	S ₂ (60)	44.67	54.83	47.86	49.47	45.80
3.	S ₃ (80)	44.95	55.83	48.12	48.93	48.2

The Best Treatment

The selection method of the best treatment was carried out based on suitability of parameters: yield, moisture content, antioxidant activity and tannin content, after ANOVA analysis. It was observed by comparing each parameter and economic value. The findings confirmed that the treatment combination with best parameters was extraction at a temperature of 40 °C for 4 hours. The best parameter of treatment is shown in Table 6.

Table 6. The parameters of the best treatment

No.	Parameter	Value
1	Yield (% dry weight)	12.20
2	Moisture Content (%)	0.023
3	Tannin content (mg GAE / g. dry weight)	424.99
4	IC50 (ppm)	42.54

Conclusions

The best combination of temperature and length of time of kinetic maceration – digestion to yield and quality extract of areca seed crude tannin was at temperature of 40 °C with extraction time of 4 hours. It produced yield of 12.20 % dry weight, moisture content of 0.023%, tannin content of 424.99 mg GAE/g dry weight and IC50 of 42.54 ppm.

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Conflict of interest

The authors declare that there is no conflict of interest in this publication.

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